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16. ABSTRACT

To determine pulmonary deposition, translocation, and clearance of inhaled fly ash, hamsters received a single 95-min nose-only exposure to neutron-activated fly ash. Over a period of 99 days postexposure, the hamsters were sacrificed in groups of six animals. Lungs, liver, kidneys, decapitated and skinned carcass, pelt, head, gastrointestinal tract, urine, and feces were collected for analysis of the radionuclide tracers ^{46}Sc, ^{59}Fe, and ^{60}Co by γ-ray spectrometry. The fly ash burden estimates as determined by the radionuclides ^{46}Sc and ^{59}Fe are in good agreement for the majority of samples analyzed. Such close agreement indicates fly ash particulate levels in the lungs, carcass, head, pelt, GI tract, and feces rather than leached radionuclides. Relative to the ^{46}Sc and ^{59}Fe-based estimates, fly ash deposition estimates obtained with the isotope ^{60}Co were appreciably lower for the lungs and appreciably higher for one or more sacrifice times for carcass, liver, head, pelt, and urine samples. This indicates that ^{60}Co (and thus the element cobalt) selectively leached from fly ash deposited in the deep lung, translocated to other sites, and was excreted in the urine. An estimated average of 63 μg fly ash, or 2 to 3% of the inhaled fly ash, was initially retained in the respiratory tract. The estimated biological half-times of the fly ash were 2.6 and 34.5 days, probably for the airways and for the deep lung, respectively. After 99 days, the mean lung burden had decreased to about 10% of its initial value.

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Fate of Inhaled Fly Ash in Hamsters[1]

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To determine pulmonary deposition, translocation, and clearance of inhaled fly ash, hamsters received a single 95-min nose-only exposure to neutron-activated fly ash. Over a period of 99 days postexposure, the hamsters were sacrificed in groups of six animals. Lungs, liver, kidneys, decapitated and skinned carcass, pelt, head, gastrointestinal tract, urine, and feces were collected for analysis of the radionuclide tracers ^{46}Sc, ^{59}Fe, and ^{60}Co by γ-ray spectrometry. The fly ash burden estimates as determined by the radionuclides ^{46}Sc and ^{59}Fe are in good agreement for the majority of samples analyzed. Such close agreement indicates fly ash *particulate* levels in the lungs, carcass, head, pelt, GI tract, and feces rather than leached radionuclides. Relative to the ^{46}Sc and ^{59}Fe-based estimates, fly ash deposition estimates obtained with the isotope ^{60}Co were appreciably lower for the lungs and appreciably higher for one or more sacrifice times for carcass, liver, head, pelt, and urine samples. This indicates that ^{60}Co (and thus the element cobalt) selectively leached from fly ash deposited in the deep lung, translocated to other sites, and was excreted in the urine. An estimated average of 63 μg fly ash, or 2 to 3% of the inhaled fly ash, was initially retained in the respiratory tract. The estimated biological half-times of the fly ash were 2.6 and 34.5 days, probably for the airways and for the deep lung, respectively. After 99 days, the mean lung burden had decreased to about 10% of its initial value.

INTRODUCTION

Inhalation exposures of laboratory animals to environmental or industrial air pollutants simulate more closely than other routes of administration the mode of human exposure to these agents. Inhalation exposures are, therefore, frequently used to determine the biological effects of an air pollutant—or a combination of air pollutants—in an animal model in an attempt to assess the risk of such exposures to man. Generally, such inhalation studies involve the exposure of animals to different dose levels of the agent(s) to be investigated, with body weight, life span, and histologic findings serving as criteria to evaluate biological effects; sometimes the criteria are extended to include biochemical, physiological, or other tests.

Regardless of the details of the experimental protocol for such studies, it is important to obtain information on the fate of the material deposited in the lungs of the exposed animals to interpret correctly results of the exposures. More specifically, it is of interest to determine that fraction of the inhaled material which is deposited in the deep lung, the biological half-life of the material in the lung, whether the material translocates from the lung to other sites, and, if so, the rate of translocation.

Pulmonary deposition, translocation, and clearance studies, however, are often problematic in that only minute quantities of material are deposited in the deep

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lung, which decrease as a function of time as the material is cleared from the lung. Usually the elements to be analyzed also occur naturally at varying levels in animal tissues, further confounding analysis. The extremely sensitive technique of neutron activating the material before the animal exposure and subsequent γ-ray analysis of the tissue and excreta samples circumvents this analytical problem as the radioactively labeled material can be readily measured against a very low background in samples from control animals.

The objective of this study was to provide supplementary and complementary information to inhalation experiments with nickel-enriched fly ash by investigating pulmonary deposition, translocation, and clearance characteristics of neutron-activated fly ash in our animal model, the hamster. Using neutron-activated fly ash for the inhalation exposure and γ-ray analysis of the tissues of interest, the fate of the material deposited in the animals was monitored for 99 days.

METHODS

Neutron activation of coal fly ash. To provide the radioactive feedstock from which the experimental aerosol was to be generated, approximately 50 g of coal fly ash[2] was prepared for neutron activation in Hanford's N-reactor. The feedstock fly ash was dried at 105°C to remove moisture and irradiated in five 10-g aliquots in the core of the reactor. Each aliquot was encapsulated in a sealed quartz container and irradiated for 2 hr in a neutron flux of approximately 10^{14} n/cm^2/sec. Radionuclides were induced by (n, γ) reactions on the major and minor elements in the fly ash. Following the irradiations, the neutron-activated fly ash was stored for a period of about 5 weeks to allow complete or partial decay of the short-lived activation products.

At the end of the decay period, the five separate fly ash aliquots were recombined and the entire inventory was sieved through an 80-mesh screen to provide a uniform particle size distribution for subsequent homogenization. A gentle hand-grinding of a few larger aggregates was required to sieve all the irradiated material. To obtain a uniform specific γ-ray activity, the fly ash was then homogenized by mixing for several hours with a wrist-arm shaker device. Replicate 0.1-g aliquots of the sieved and homogenized fly ash were then sampled and counted on a solid-state Ge(Li) detector to verify a homogeneous specific γ-ray activity and to identify potential isotopes which could be used as tracers. Because of their intensities, relatively long half-lives, and other favorable radioactive decay properties, the radionuclides ^{60}Co, ^{46}Sc, and ^{59}Fe were selected as the preferable fly ash tracers. The elemental concentrations of cobalt, scandium, and iron in the feedstock fly ash were determined by instrumental neutron activation analysis and were found to be 39 ppm, 18.5 ppm, and 20% by weight, respectively. Following these preparations and evaluations, the irradiated fly ash was aerosolized for the animal exposure.

Aerosol exposure system. Exposure of the hamsters to the neutron-activated fly ash took place in our radioactive aerosol exposure laboratory. The 20-liter aerosol exposure chamber (Wehner *et al.*, 1977) was constructed of Lucite with exposure ports for nose-only exposures arranged in seven tiers, each containing 10 expo-

[2] Prepared by Battelle Columbus Laboratories from Ohio coal, mined in Pittsburgh No. 8 seam.

sure ports. For exposure, the animals were placed in soft-drink bottles that had the bottom and part of the top removed so that the noses of the animals were close to the open tops of the bottles; the bottles were inserted through neoprene stoppers into the ports of the exposure chamber. The animals were maintained in this position by wadding pushed against their posteriors, and by neoprene stoppers taped to the bottoms of, and sealing, the bottles. The hamsters and four aerosol samplers were positioned on the lower six of the seven tiers. More specifically, three filter holders with Metricel DM-450 filters (0.45 μm mean pore size) were positioned on tiers 2, 4, and 6. The filters were weighed before and after sampling to estimate total aerosol concentration. An Andersen cascade impactor (Andersen, 1966) was positioned on tier 3 to allow estimations of the particle size distribution and the respirable aerosol concentration. The ports not occupied by animals or aerosol samplers were closed with neoprene stoppers.

The fly ash aerosol was generated by a Wright Dust Feed Mechanism (Wright, 1950) and then passed through a cyclone elutriator for removal of particles larger than approximately 10 μm aerodynamic equivalent diameter (AED). The aerosol exposure chamber, aerosol generating equipment, and aerosol sampling devices were contained in a Lucite glove box under slight negative pressure relative to the exposure room.

Animal exposure, serial sacrifices, and postmortem procedures. Sixty 5-month-old male Syrian golden hamsters (*Mesocricetus auratus*, outbred LAK:LVG, Charles River Lakeview Laboratories), weighing from 120 to 130 g, were prepared for nose-only exposure to the aerosol of neutron-activated fly ash as described. One hamster died in the restraint tube before exposure; another died during the 95-min exposure, leaving a total of 58 animals to complete the exposure. The animals were of the same strain and were obtained from the same source as those in the aforementioned chronic inhalation study with nickel-enriched fly ash. The aerosol data are summarized in Table 1. The mean ± standard deviation

TABLE 1
SUMMARY OF NEUTRON-ACTIVATED FLY ASH AEROSOL DATA

Isotope	Energy (MEV)	AMAD[a] (μm)	GSD[b]
^{233}Pa	0.312	3.53	2.75
^{51}Cr	0.320	2.81	2.84
^{58}Co	0.811	3.68	2.82
^{46}Sc	0.899	3.54	2.77
^{59}Fe	1.099	4.00	2.78
^{46}Sc	1.121	3.54	2.71
^{60}Co	1.173	3.38	2.77
^{59}Fe	1.292	3.99	2.80
^{60}Co	1.332	3.40	2.77
^{152}Eu	1.408	3.33	2.70
Total counts		3.63	2.79
Mean ± standard deviation		3.53 ± 0.33	2.77 ± 0.05

[a] The activity median aerodynamic diameter (AMAD) was determined by means of a cascade impactor.

[b] GSD = Geometric standard deviation.

of the respirable aerosol concentration was 470 ± 39 μg/liter; the activity median aerodynamic diameter was approximately 3.5 ± 0.33 μm, and the geometric standard deviation 2.8 ± 0.045.

After exposure, the animals were serially sacrificed in groups of six by randomized selection of one animal from each of the six occupied tiers of the exposure chamber. Tissues and excreta were collected 15 min, 1 day, 3, 7, 14, 24, 51, and 99 days after exposure. The lungs, including mediastinal tissue, liver, kidneys, skinned and decapitated carcass, pelt, head, gastrointestinal (GI) tract, urine, and feces were analyzed for radioactive isotopes by γ-ray spectrometry, and the γ-ray counts were compared with those obtained from 10 unexposed control hamsters. Five of the control animals were killed 2 days after the test animals were exposed; the remaining five control animals were killed 102 days after exposure of the test animals. The tissues from the control animals were analyzed like those from the exposed animals, together with urine and feces samples collected during the 2 days preceding the first sacrifice.

Analyses were carried out on the lungs to determine pulmonary deposition and clearance, and on liver and kidneys to show whether during translocation (if any) and clearance of pulmonary deposits of fly ash, the material would pass through and/or accumulate in these organs. The remainder of the skinned carcass was analyzed for fly ash with no attempt at further differentiation of translocation sites. The skin was removed from the carcass to reduce the chance of sample contamination. The head was analyzed separately to prevent the expected relatively heavy deposition on and in the head area from confounding the other deposition data. Analyzing excreta as part of a translocation and clearance study sheds light on the excretion rate of the material and aids in estimating initial deposition of the inhaled material.

Analysis of samples. Tissue samples for γ-ray analysis were preserved by freezing. The small tissue samples (lung, kidneys, and liver) and fecal samples were transferred directly into vials for counting. Large samples (carcass, head, GI tract, and pelt) required high-temperature ashing to reduce the volume for analysis. Previous work in our laboratory has shown that ashing does not cause losses of the radionuclides through volatilization (L. A. Rancitelli, unpublished data, 1975). The urine samples were transferred into polyethylene vials and evaporated to dryness with a heat lamp before analysis. The prepared samples were centered in 2-dram polyethylene vials for γ-ray counting.

The γ-ray analyses were performed on a Ge(Li) detector and several Na(Tl) γ-γ coincidence spectrometers. Feedstock fly ash standards in geometrical arrangements identical to those of the samples were counted on each detector system used for sample analysis. Counting times ranged from 30 to 2000 min, depending on the activity in the samples. The amount of fly ash in the samples was determined by comparing the samples with the feedstock standards.

The isotope ^{60}Co was used in all cases to calculate the fly ash in samples since it has a long half-life (5.2 years as compared to 83.8 days for ^{46}Sc and 45 days for ^{59}Fe) and easily detectable coincident γ-rays. For the period of this study, the decay correction for the ^{60}Co activity was 5% or less. In addition, ^{46}Sc and ^{59}Fe activities were used to estimate the quantity of fly ash for purposes of comparing the ^{46}Sc-based and ^{59}Fe-based values with the ^{60}Co-based values.

The advantage of using more than one radioactive tracer to monitor the clearance and translocation of fly ash lies in observing two or more elements with different dissolution and transfer coefficients in a biological system. For example, if the ^{60}Co:^{46}Sc activity ratio in a hamster tissue is identical to the ^{60}Co:^{46}Sc ratio in the inhaled fly ash aerosol, it strongly suggests that the pair of radioisotopes represent particulate fly ash in the tissue. Radioisotopes which have been extracted or leached from the inhaled fly ash do not show the characteristic activity ratios of particulate fly ash. Indeed, a situation that would produce the same ^{60}Co:^{46}Sc ratio in tissue as was observed in the fly ash would require that these radionuclides be leached, absorbed, and transferred in identical fashion. This possibility is unlikely and is not supported by past experience in our laboratory which suggests that biological systems take up Co in preference to Sc (L. A. Rancitelli, unpublished data, 1975).

Relative variations of tracer radionuclide specific activities as related to particle size. The elemental composition of coal fly ash is known to vary considerably as a function of particle size. For example, the abundance of several volatile trace metals has been shown to be highly enriched in the smaller particle size ranges (Campbell *et al.*, 1978). Because of this, the specific activity of a given radionuclide tracer in the irradiated feedstock may be appreciably different from the specific activity of the same radionuclide in the experimentally generated aerosol or fractions thereof. These differences may result from: (1) changes in particle size distributions caused by the aerosol generating system (e.g., by the elutriator); and/or (2) particle size separations in the respiratory system of the experimental animals.

In this study, the measurement of deposited fly ash particulates was to be quantified by comparison to the known specific activities of the sieved and homogenized feedstock. However, if during aerosol generation or inhalation the particle size distribution changed, the original elemental abundances of cobalt, iron, and scandium in the prepared feedstock fly ash might also be changed and thus compromise the normalization to the "standard" specific activities of the feedstock samples. Therefore, the possible differences in specific activities for the feedstock and derivative aerosol fractions needed to be evaluated to preserve the accuracy of the intended quantitative analysis.

To resolve this potential problem, samples of the experimentally derived fly ash aerosol were collected on preweighed 0.45-μm Millipore filters and on preweighed stages of an Andersen cascade impactor. The aerosolized fly ash material that was collected was weighed and then counted on the same Ge(Li) detector that had been used previously for the 0.1-g feedstock standards. The resulting data, including those of the feedstock standards, are tabulated in Table 2.

To evaluate the specific counting activity of each radionuclide in the experimental aerosol relative to its activity in the original feedstock fly ash, ratios were computed using the mean values in Table 2. The measure of difference was calculated as the ratio of the sum of means for the two energies associated with a given isotope for the aerosol (or indicated fraction) to the sum of means for the feedstock standard. The calculated ratios are given in Table 3. Estimates of the standard deviation of these ratios were approximated by propagation of error techniques, and approximate 95% confidence bounds were calculated for each

TABLE 2

SPECIFIC COUNTING ACTIVITIES (±1 σ COUNTING ERRORS)[a] OF FEEDSTOCK AND AEROSOL FLY ASH SAMPLES (CPM/g)

Material	Impactor stage	Size range (μm)	Isotope and energy (MEV) ^{60}Co 1.173	^{60}Co 1.132	^{59}Fe 1.099	^{59}Fe 1.292	^{46}Sc 0.889	^{46}Sc 1.121
Feedstock fly ash standard Mean (±SD)			3720 ± 66	3290 ± 16	26,670 ± 160	17,690 ± 140	26,850 ± 330	21,680 ± 270
Fly Ash Aerosol Mean (±SD)			3970 ± 190	3460 ± 190	24,100 ± 110	15,940 ± 280	26,550 ± 500	21,110 ± 130
Fractionated fly ash aerosol								
	#4	3.12–4.80	3540 ± 73	3190 ± 65	23,320 ± 180	15,320 ± 140	24,420 ± 210	19,550 ± 170
	#5	2.41–3.12	3490 ± 63	3070 ± 57	18,920 ± 150	12,530 ± 120	22,280 ± 180	18,140 ± 150
	#6	0.99–2.41	3390 ± 78	2980 ± 69	17,100 ± 170	11,270 ± 140	21,790 ± 220	17,370 ± 190
	#7	0.64–0.99	3420 ± 84	2970 ± 75	16,350 ± 190	10,990 ± 150	21,700 ± 230	17,330 ± 200
	#8	0.43–0.64	3920 ± 56	3480 ± 51	17,160 ± 120	11,320 ± 93	22,630 ± 150	18,040 ± 130
	#9	<0.43	3550 ± 82	3130 ± 74	15,070 ± 170	10,420 ± 140	21,510 ± 220	17,530 ± 190
Mean #4 through #9 (±SD)			3550 ± 190	3140 ± 190	17,990 ± 2900	11,980 ± 1780	22,390 ± 1080	17,990 ± 830

[a] Calculated as gross count‡ net count; gross count = total count, net count = gross count minus background count.

TABLE 3
RATIO (±SD) OF TRACER RADIONUCLIDE COUNTING ACTIVITIES IN FLY ASH AEROSOL TO FEEDSTOCK FLY ASH

Ratio	^{46}Sc	^{59}Fe	^{60}Co
Sub-4.8 μm fraction/feedstock	0.83[a] ± 0.040	0.68[a] ± 0.105	0.96 ± 0.054
Total aerosol/feedstock	0.98 ± 0.024	0.90[a] ± 0.010	1.06[a] ± 0.026

[a] An approximate 95% confidence interval does not include the exptected ratio of 1.0.

ratio. The ratios for which the confidence interval did not include 1.0 (equality of activity in aerosol and feedstock) are indicated.

These results imply that: (1) the respirable fraction of the aerosol (<4.8 μm) had a significantly lower specific activity for the radionuclides ^{46}Sc and ^{59}Fe; (2) the total aerosol had significantly higher and lower specific activities for ^{60}Co and ^{59}Fe, respectively. These variations are due to the anticipated changes in elemental concentrations as a function of particle size. To correct for their source of error in each radionuclide tracer, the ratios in Table 3 were applied as corrective factors to ensure an accurate quantitative analysis of deposited fly ash particles as measured by each isotope. The ratios representing the respirable fraction were applied to the lung tissue samples. The ratios associated with the unfractionated or "whole" fly ash aerosol were applied to all other animal tissue and excreta samples.

RESULTS AND DISCUSSION

The fly ash burdens as a function of time in the tissues and excreta of the exposed hamsters and the background values for the control animals are tabulated in Table 4.

All values for the exposed animals represent the mean ± one standard deviation for six hamster samples. The sizable standard deviations reflect the large degree of scatter between the individual values within each group of animals. However, in spite of the magnitude of these deviations, examination of the ^{46}Sc-, and ^{59}Fe-, and the ^{60}Co-based lung values shows good agreement between the ^{46}Sc- and the ^{59}Fe-based data. This agreement is shown in Table 5 by a sample listing of the six individual lung burden analyses for the 15-min sacrifice. The measurement precision for each individual value in Table 5 is 1 to 10% and the good agreement between values based on ^{46}Sc and ^{59}Fe indicates that these tracers remain together in and represent fly ash *particles* rather than leached radionuclides.

The ^{60}Co-based lung values, on the other hand, are substantially lower than those based on the other two radionuclides. This suggests that ^{60}Co leached selectively from the fly ash deposited in the deep lung, translocating primarily to the head and the pelt, as indicated by the consistently and markedly higher ^{60}Co values for these samples. Previous work in our laboratory with similar findings (Wehner *et al.*, 1977; Wilkerson *et al.*, 1977), significantly higher ^{60}Co values for the urine samples, and the increased ^{60}Co values for the head and pelt support the statement that leaching of ^{60}Co occurred. Apparently, leaching and translocation of ^{60}Co from the lung occurred rapidly as indicated by the high ^{60}Co values for the

TABLE 4

FLY ASH BURDENS IN TISSUES AND EXCRETA AS A FUNCTION OF TIME AFTER EXPOSURE[a,b,c]

Time of sacrifice after exposure	^{46}Sc	^{59}Fe	^{60}Co	^{46}Sc	^{59}Fe	^{60}Co	^{46}Sc	^{59}Fe	^{60}Co
	Lungs			Liver			Kidneys		
15 min	65.8 ± 49.3	63.2 ± 48.9	40.1 ± 33.1	0.52 ± 0.45	1.2 ± 1.0	3.4 ± 1.9	<0.12 ± 0.07	<0.40 ± 0.06	<0.94 ± 0.48
1 day	48.8 ± 15.2	48.4 ± 13.6	23.0 ± 7.0	0.41 ± 0.19	2.7 ± 1.9	1.9 ± 0.59	0.18 ± 0.09	<0.51 ± 0.05	0.49 ± 0.21
3 days	55.7 ± 28.3	55.4 ± 26.0	25.6 ± 13.6	0.62 ± 0.27	3.1 ± 1.1	1.6 ± 0.54	<0.25 ± 0.12	0.43 ± 0.12	0.43 ± 0.12
7 days	27.9 ± 10.6	29.0 ± 11.6	11.5 ± 5.5	0.35 ± 0.17	2.5 ± 1.2	1.0 ± 0.50	0.13 ± 0.09	<0.65 ± 0.08	0.38 ± 0.14
14 days	34.1 ± 16.5	37.3 ± 16.7	14.1 ± 7.2	0.53 ± 0.26	4.1 ± 1.8	0.86 ± 0.27	<0.19 ± 0.09	<0.85 ± 0.43	<0.28 ± 0.10
24 days	20.7 ± 9.3	24.2 ± 10.3	8.3 ± 4.4	0.48 ± 0.24	2.9 ± 1.2	0.79 ± 0.34	0.08 ± 0.03	<1.2 ± 0.45	<0.18 ± 0.04
51 days	12.2 ± 10.3	14.5 ± 11.5	5.2 ± 5.0	0.59 ± 0.50	<2.2 ± 0.99	0.48 ± 0.42	<0.09 ± 0.03	<1.3 ± 0.15	<0.20 ± 0.04
99 days	6.4 ± 4.4	<7.65 ± 5.5	2.4 ± 1.7	0.36 ± 0.24	<2.6 ± 1.2	<0.21 ± 0.09	<0.21 ± 0.31	<2.3 ± 0.23	<0.17 ± 0.05
Controls									
2 days	<0.07 ± 0.03	<1.2 ± 0.13	<0.21 ± 0.09	<0.04 ± 0.004	<0.75 ± 0.22	<0.16 ± 0.04	<0.04 ± 0.02	<0.78 ± 0.08	<0.16 ± 0.05
102 days	<0.09 ± 0.03	<3.6 ± 1.6	<0.14 ± 0.08	<0.05 ± 0.02	<3.8 ± 1.3	<0.13 ± 0.02	<0.07 ± 0.02	<2.6 ± 1.2	<0.15 ± 0.03
	Carcass			GI tract			Head		
15 min	26.9 ± 13.0	28.0 ± 14.6	66.9 ± 26.1	814 ± 244	792 ± 233	767 ± 212	102 ± 57.2	103 ± 50.4	120 ± 55.3
1 day	10.0 ± 8.2	11.4 ± 7.9	14.9 ± 9.7	259 ± 108	228 ± 140	270 ± 106	20.3 ± 10.8	21.8 ±10.6	46.5 ± 25.7
3 days	6.0 ± 3.4	0.0 ± 3.6	8.8 ± 2.8	57.6 ± 55.4	59.6 ± 57.3	67.6 ± 52.0	6.1 ± 2.6	9.2 ± 3.4	25.7 ± 19.3
7 days	1.7 ± 1.7	4.0 ± 1.3	2.4 ± 1.1	<4.3 ± 1.1	<5.3 ± 1.6	<11.3 ± 3.3	2.0 ± 1.1	4.7 ± 2.7	22.0 ± 12.8
14 days	1.5 ± 0.84	5.7 ± 2.3	2.2 ± 0.74	4.7 ± 0.71	<5.7 ± 2.4	<5.8 ± 2.1			
24 days	<0.82 ± 0.49	<4.5 ± 1.5	1.3 ± 0.75						
51 days	<0.61 ± 0.54	<3.6 ± 2.0	<0.67 ± 0.54						
99 days	0.70 ± 0.37	<4.3 ± 1.3	0.54 ± 0.30						
Controls									
2 days	<0.08 ± 0.02	<0.86 ± 0.10	<0.27 ± 0.08	<1.6 ± 0.41	<2.0 ± 0.93	<3.4 ± 1.2	<0.08 ± 0.01	<0.81 ± 0.20	<0.26 ± 0.07
102 days	<0.10 ± 0.02	<3.2 ± 1.2	<0.19 ± 0.04						

	Pelt			Feces			Urine		
15 min	80.6 ± 39.1	75.8 ± 37.9	91.6 ± 43.6						
1 day	23.5 ± 12.6	21.5 ± 12.2	34.1 ± 17.2	873 ± 340	831 ± 327	903 ± 127	1.0 ± 0.7	3.2 ± 0.33	21.3 ± 10.6
3 days	8.8 ± 2.3	9.2 ± 2.6	37.9 ± 30.0	483 ± 358	471 ± 349	464 ± 306	0.88 ± 0.74	<3.2	16.8 ± 14.2
7 days	2.9 ± 2.0	4.4 ± 2.4	18.0 ± 14.3	127 ± 168	130 ± 173	142 ± 180	0.9 ± 1.6	<3.5 ± 0.18	3.8 ± 4.2
14 days									
24 days									
51 days									
99 days									
Controls	<0.08 ± 0.02	<0.70 ± 0.05	<0.26 ± 0.10	<2.4 ± 1.3	<4.2 ± 1.5	<6.8 ± 4.3	<0.05 ± 0.02	<4.2 ± 0.22	<0.13 ± 0.01

[a] All values are in micrograms and represent the mean ± 1 SD of six hamster samples.

[b] The standard deviations associated with means reported as "less than" were estimated on limited (<) data and are, therefore, only approximate. If any individual value upon which the mean is based is a limited number (<), the mean is reported as a limited (<) number.

[c] The differences in the means reported as "less than" (ranging from <11.3 [GI tract; ^{60}Co 7-day postexposure value] to 0.5 [^{46}Sc value for urine of controls]) are mainly due to different counting times, resulting in different sensitivities.

TABLE 5
INDIVIDUAL HAMSTER LUNG BURDEN ESTIMATES FOR THE 15-MIN SACRIFICE[a]

Sacrifice time postexposure	Tier number	Hamster number	Lungs		
			^{46}Sc	^{59}Fe	^{60}Co
15 min	2	5	45.7	43.2	27.0
	3	16	36.4	34.6	21.7
	4	23	153	152	101
	5	32	44.4	41.7	23.9
	6	47	20.9	20.4	12.2
	7	59	94.0	87.7	54.9
Mean ± SD			65.7 ± 49.3	63.2 ± 48.9	40.1 ± 33.1

[a] Estimated as micrograms fly ash retained in lung.

first (15-min postexposure) sacrifice samples for kidneys, liver, and carcass. The leached ^{60}Co was excreted in the urine, and most of the urinary ^{60}Co excretion took place within the first few days after exposure.

Figure 1 shows the pulmonary deposition and clearance of the fly ash, based on the data from ^{46}Sc. The estimated curve is within one standard error of the mean (SEM) for all sacrifice times except 7 days (at 7 days the predicted value is within 2 SEM), indicating a good fit to the data. A nonlinear algorithm was used to fit a

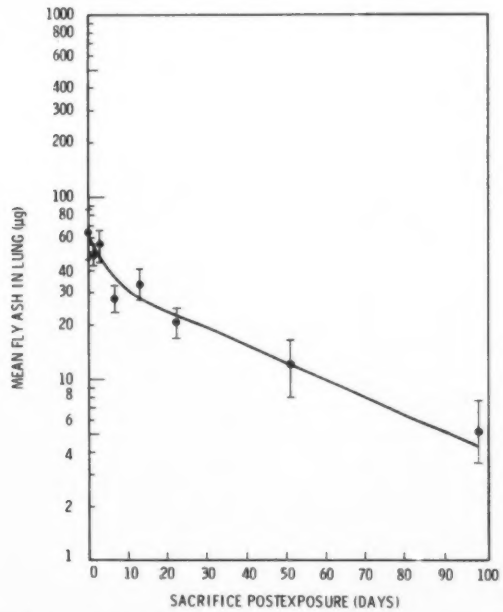

FIG. 1. Estimated pulmonary clearance of inhaled fly ash in hamsters (mean ± SEM of six animals per sacrifice time) based on ^{46}Sc data.

two-compartment model to the ^{46}Sc and the ^{59}Fe-based lung burdens in Table 4 as a function of time. The fits were preferable over their single compartment counterparts by minimizing the residual sum of squares. Similarly, for ^{46}Sc, the fit was slightly preferable to the ^{59}Fe modeling. The function for ^{46}Sc was

$$Y = 26.5\, e^{-0.268t} + 36.7\, e^{-0.0201t}. \quad (1)$$

This model estimates the biological half-times to be 2.6 ± 3.2 and 34.5 ± 28.7 days (mean ± standard deviation), the sizable standard deviations reflecting the variability in the data. The shorter biological half-time of 2.6 days probably reflects material cleared relatively quickly by mucociliary action, whereas the half-time of 34.5 days reflects the deep lung burden (alveolar deposition). At 99 days postexposure, the mean lung burden had decreased to about 10% of its initial value. Extrapolating from (1), it can be estimated that near-complete clearance and/or translocation of fly ash from the lung would have been achieved approximately 200 days postexposure, at which time only about 1% of the lung burden is predicted to be retained in the lung. From (1), an estimate of the initial lung burden is 63.2 ± 8.3 μg.

The percentage of inhaled fly ash that was deposited and retained in the respiratory tract may be estimated from the following known parameters: respirable aerosol concentration (470 μg/liter); exposure time (95 min); minute volume of the hamster (60 ml/min[3] [Gleysteen and Stroud, 1974]); and the quantity of fly ash deposited in the respiratory tract of the exposed animals (~63 μg). These calculations indicate that approximately 2.7 mg of fly ash was inhaled during the 95-min exposure. Of this quantity, about 63 μg, or 2.3%, was retained. This estimate is in good agreement with estimates of slightly less than 2% retention of inhaled nickel-enriched fly ash during the 6-hr acute toxicity test at a respirable aerosol concentration = 220 μg/liter, and of between 3 and 4% during the forty 6-hr exposures (respirable aerosol concentration ~190 μg/liter) of the subacute toxicity test (Wehner *et al.*, 1979).

Results of the acute toxicity test showed no appreciable decline in lung burden between 7 days (~ 78 μg) and 30 days postexposure, which might suggest a rather long biological half-time of nickel-enriched fly ash retained in the hamster lungs (Wehner *et al.*, 1979). However, the 7-day value of 78 μg was based on only two animals. The present data indicate biological half-times of 2.6 and 34.5 days for fly ash, probably deposited in the airways and in the deep lung, respectively. Available evidence suggests that there should be no difference in pulmonary deposition, retention, and clearance characteristics of fly ash particles and nickel-enriched fly ash particles (Wehner *et al.*, 1979). Given the larger data base for the results of this pulmonary clearance study, it is reasonable to accept as valid the half-time values of 2 to 3 days for the airways and about 35 days for the deep lung.

The head samples showed a deposition of more than 100 μg fly ash. This quantity combines external deposition on the head and on or about the nares, and internal deposition in the nasopharyngeal area, plus some material probably in the process of being cleared by mucociliary action from the lower airways. This

[3] Not allowing for possible changes in breathing pattern due to confinement of the animals in the restraint tubes during the 95-min exposure.

appreciable quantity, comprising both externally and internally deposited fly ash in an indistinguishable manner, would have confounded the carcass and pelt deposition data. For this reason the head was analyzed separately. Within 7 days after exposure, the head sample values had decreased to less than 5% of their original values as indicated by the ^{46}Sc and ^{59}Fe data. The elevated ^{60}Co data, whose difference from the ^{46}Sc and ^{59}Fe data increased as a function of time, can again be attributed to leaching of ^{60}Co from the fly ash. The reasons for the preferred translocation of ^{60}Co to the head—and also to the pelt—are not known. Initially high deposition due to the head's exposed position during exposure, and subsequent rapid clearance due to natural clearance mechanisms and the animals' grooming themselves, was expected.

What has been said about the head samples holds largely true for the pelt samples, too.

The deposition in the liver samples from the exposed animals was compared to control animals at both sacrifice times by a two-tailed Mann–Whitney U test. It was assumed that the estimated contents could be ranked by the reported measurements, even though some values are reported as upper limit values. In the case of ^{59}Fe, the control estimates at 102 days were considerably higher than at 2 days. The increased detection limit at 102 days is due to the radioactive decay of the tracer radionuclide ^{59}Fe, i.e., the detection limit increased by $(1/e^{-\lambda t})$[4] or approximately a factor of 5 after 102 days. Because the detection limit is increasing as a function of time, the exposed liver analyses based on ^{59}Fe are at or near the detection limit of the system. Thus, it can be concluded that the ^{59}Fe tracer data do not necessarily support the hypothesis that fly ash translocated to the liver.

The ^{46}Sc- and ^{60}Co-based fly ash estimates for the liver samples of exposed hamsters were generally higher than those for both groups of controls, which were similar to one another. However, because of lack of agreement, these elevated levels cannot be considered as a definitive verification of fly ash particles. The higher values for ^{60}Co (relative to ^{46}Sc) reflect the presence of leached ^{60}Co. In both the liver and kidneys, the ^{60}Co values were highest for the first sacrifice and decreased steadily thereafter until they approached control levels. This suggests passage of leached ^{60}Co through these organs and is further evidence for the selective leaching of ^{60}Co from fly ash particles deposited in the hamster.

The rather constant and very small quantities of ^{46}Sc in the liver samples of the exposed animals represent less than 0.1% of the available ^{46}Sc deposited in the animals. These small quantities of ^{46}Sc could represent fly ash particulates, but this conclusion lacks verification by a second independent isotope. The estimates based on ^{46}Sc probably represent small quantities of leached ^{46}Sc or unverifiable fly ash particulate levels of approximately 0.5 μg total.

The deposition in the kidneys was not treated statistically due to the large number of values which were reported only as an upper limit and because the amounts in the exposed animals were so similar to the values observed in the control animals (^{60}Co data excluded).

[4] $e^{-\lambda t}$ represents the decay function for a radioactive species. For ^{59}Fe, $\lambda = 0.693/45$ days, and t, the elapsed time, = 102 days.

The most plausible explanation for the values from the skinned carcass (minus head, liver, kidneys, and GI tract) being higher than the control values for the first few sacrifices is the external deposition of fly ash particles on feet and on or between toes from where they were not removed with the pelt. The mean for the 15-min sacrifice ^{60}Co values is more than twice as high as the means of the ^{46}Sc and ^{59}Fe samples. This might reflect the rapid leaching of ^{60}Co from the fly ash in the lung and subsequent translocation to the carcass.

Considerable quantities of fly ash were found in the GI tracts of the first groups sacrificed. The means (^{46}Sc and ^{59}Fe data only) decreased from roughly 800 μg to 250, 60, and 5 μg for the 15-min, 1-day, 3-day, and 7-day sacrifices, respectively. These values combine material initially deposited in the respiratory tract and subsequently translocated by mucociliary clearance mechanisms to the GI tract, and externally deposited fly ash which was translocated to the GI tract by grooming (licking and swallowing) of the animals after exposure.

The values for the fecal samples reflect the GI burdens, decreasing (^{46}Sc and ^{59}Fe data only) from about 850 μg to 480 and 130 μg for the day 1, day 3, and day 7 samples, respectively. As can be expected for fecal samples, the values varied considerably about the means as indicated by the large standard deviations. The importance of the urine data lies in the high ^{60}Co values, indicating selective leaching of ^{60}Co from the fly ash particles, as pointed out before.

In summary, the fly ash burden estimates as determined by the radionuclides ^{46}Sc and ^{59}Fe are in good agreement for the majority of samples analyzed. Such close agreement indicated fly ash *particulate* levels in the lungs, carcass, head, pelt, GI tract, and feces rather than leached radionuclides. Relative to the ^{46}Sc- and ^{59}Fe-based estimates, fly ash deposition estimates obtained with the isotope ^{60}Co were appreciably lower for the lungs and appreciably higher for one or more sacrifice times for carcass, liver, head, pelt, and urine samples. This indicates that ^{60}Co (and thus the element cobalt) selectively leached from fly ash deposited in the deep lung, translocated to other sites, and was excreted in the urine. Most of the urinary ^{60}Co excretion took place within the first few days after exposure.

An estimated average of 63 μg fly ash, or 2 to 3% of the inhaled fly ash, was initially retained in the respiratory tract. The estimated biological half-times of the fly ash were 2.6 and 34.5 days, probably for the airways and for the deep lung, respectively. After 99 days, the mean lung burden had decreased to about 10% of its initial value. Extrapolating from the clearance data, it can be estimated that near-complete clearance of fly ash from the lung would have been achieved approximately 200 days postexposure.

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